HETEROPLOIDY IN TRITURUS TOROSUS. II. THE INCIDENCE OF CHROMOSOMAL VARIATIONS IN SHIPPED LARVAE [1]

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Studies on the incidence of heteroploidy and other cytological abnormalities in salamander larvae have been given considerable impetus in recent years by the work of Fankhauser and his students. Such studies have been concerned with the experimental production of chromosomal aberrations, as well as with their spontaneous occurrence in natural populations of amphibian larvae. A general review of the results obtained before 1945 is presented by Fankhauser (1945).

The eggs of the California newt, *Triturus torosus*, are laid in "clutches" which are available in large numbers near Stanford University. Since no systematic effort had been made to determine the spontaneous incidence of heteroploidy in this form, it was decided in 1947 to undertake such a survey. Accordingly, a number of egg masses were sent by Railway Express from Palo Alto to Chapel Hill, in a thermos jug containing ice and water; this is the usual method for shipping amphibian eggs. Cytological examination of the larvae developing from these eggs revealed a high frequency of chromosomal mosaicism and very radical mitotic anomalies.

A comprehensive study was begun early in 1948 in order to eliminate the possibility that such aberrations might be present in material not subjected to shipment. Tail-tips from larvae fixed in California had only a very low incidence of abnormality, suggesting that factors involved in shipment might be responsible for the observed effects. During the period when this control material was being fixed and studied in California, another group of *T. torosus* eggs was shipped east to Chapel Hill. Egg clutches collected at the same time and in the same locale as these "1948 shipped eggs" were raised under known conditions, so that a specific control for this second group of shipped larvae was available. The data from a study of the control animals have been presented in detail by Costello and Henley (1949).

The present paper will describe the results of a study of the original (1947) group of shipped larvae, as well as the second (1948) group.

Preliminary reports concerning small random samples of these shipped larvae have been noted in abstracts (Costello and Henley, 1947a, 1947b, 1948; Costello, 1948).

We are indebted to Dr. Victor C. Twitty and Dr. H. E. Lehman for their kindness in sending us the original batch of shipped eggs in 1947.

Methods

1. 1947 material. Unfortunately, no data are available concerning the details of collection and shipment of these eggs. It is probable that they were in quite advanced stages of development at the time of shipment, since most of the embryos were neurulae and tail-buds when received in Chapel Hill approximately one week later. At the time of receipt, all ice in the thermos jug had melted, but the water was still cool.

Upon their arrival in Chapel Hill, the egg clutches were placed in large finger-bowls of spring water and kept in a cold room at approximately 13° C., until the time of the first tail-clipping. The eggs were removed from the cold room and allowed to return to laboratory temperature from 1 to 24 hours prior to the amputation of the tail. After this initial clipping, they were raised at laboratory room temperature, two per dish, in small fingerbowls of spring water.

At least two photographs were made of each larva, to serve as records of their appearance and gross characteristics. MS–222 or chloretone (both made up 1 : 2000 in distilled water) was used to immobilize the larvae for photographing and for the subsequent tail-clipping. The general technique utilized for amputating and staining the tail-tips has been described earlier (Costello and Henley, 1949). In many cases, three successive tips were obtained from each individual and prepared for cytological study; they were clipped at 10 to 14 day intervals. All the animals in this group were fed cladocera and Tubifex. Four hundred fifty-nine "first" tips, 338 "second" tips and 302 "third" tips were studied from these individuals.

2. 1948 material. The 1948 larvae were derived from eggs collected at Los Trancos, California, as late cleavage stages and blastulae. Fifty clutches were shipped from Palo Alto to Chapel Hill the day following collection, in "heavily iced" thermos jugs of lake water (15 ice cubes + 500 cc. lake water). Fifty other clutches collected at the same time and in the same locale were maintained at 12° to 15° C., and were kept as specific controls for the shipped eggs.

When the eggs arrived in Chapel Hill one week after shipment, they were placed in large fingerbowls of spring water. Some of the embryos were raised at laboratory room temperature, and some in the cold room, at about 6° C. After photography and the initial tail-clipping, they were assigned serial numbers and kept individually in small fingerbowls of spring water. The regenerated tail-tip and, in some cases, a "third" tip (second regenerate) were clipped and fixed at approximately 12 to 14 day intervals. Cladocera and Enchytrea were used as food for these larvae. Three hundred seventy-nine "first" tips," 269 "second" tips, and 54 "third" tips from the 1948 shipped animals were studied cytologically.

All tail-tips from both the 1947 and 1948 groups of larvae were examined, using the procedure outlined earlier (Costello and Henley, 1949). Each tip was first studied at a magnification of 210 ×; nuclear size outlines were then drawn with the aid of the camera lucida, using 10 × oculars and an oil immersion objective. Wherever possible, the number of nucleoli in each nucleus drawn was determined in the 1948 tips; the possible value of this criterion had not been foreseen at the time when the 1947 tips were studied. Chromosome counts were established using 15 × compensating eyepieces, an oil immersion objective and the camera lucida (total magnification 2100 ×).

The tips were classified utilizing the criteria discussed by Costello and Henley (1949) : 1) Nuclear size and nucleolar number ; 2) the presence of abnormal mitotic configurations ; and 3) the presence of heteroploid metaphases checked, whenever possible, by chromosome counts. In many cases, more than one indication of abnormality was observed ; under such circumstances classification was based on the most radical and clearly demonstrable of the atypical features.

RESULTS

A summary of the data concerning shipped and control larvae is contained in Table I.

TABLE I

The incidence of cytological abnormality in shipped Triturus torosus larvae

Clipping	Total no. tips	Normal diploids		Variable nuclei		Abnormal mitoses		Mosaics	
1947 "First" tips	459	215	46.8%	213	46.4%	21	4.6%	10	2.2%
1948 "First" tips	379	166	43.8%	146	38.5%	54	14.2%	13	3.4%
Total	838	381	45.5%	359	42.8%	75	8.9%	23	2.7%
1948 Control "First" tips	582	423	72.6%	105	18.0%	53	9.1%	1	0.17%
1947 "Second" tips	338	142	42.0%	81	23.9%	39	11.5%	76	22.5%
1948 "Second" tips	269	98	36.4%	107	39.8%	55	20.4%	9	3.3%
Total	607	240	39.5%	188	30.9%	94	15.5%	85	14.0%
1948 Control "Second" tips	429	284	66.2%	88	20.5%	55	12.8%	2	0.5%
1947 "Third" tips	302	138	45.7%	126	41.7%	27	8.9%	11	3.6%
1948 "Third" tips	54	15	27.8%	33	61.1%	5	9.3%	1	1.8%
Total	356	153	43.0%	159	44.6%	32	9.0%	12	3.4%

It is apparent that the incidence of all types of abnormality is roughly comparable among the first and third tips. Furthermore, the frequency of atypical features in first and third tips of both groups of shipped larvae and in the control larvae is not nearly so pronounced as in the second tips. It has previously been pointed out that second tips are far superior to both first and third tips for cytological study (Costello and Henley, 1949), and that among control animals the occurrence of chromosomal aberrations is much more frequent in second tips. The somewhat greater incidence of abnormal mitoses among control first tips (9.1%) than in the first tips of shipped material (8.9%) is surprising. However, this apparent discrepancy can perhaps be explained by the fact that the 1948 control tips were clipped at a slightly later time than the shipped tips. They are characterized in general by a somewhat larger number of mitoses, increasing the chances that abnormalities would be noted. Among the second tips, the occurrence of cytological abnormality
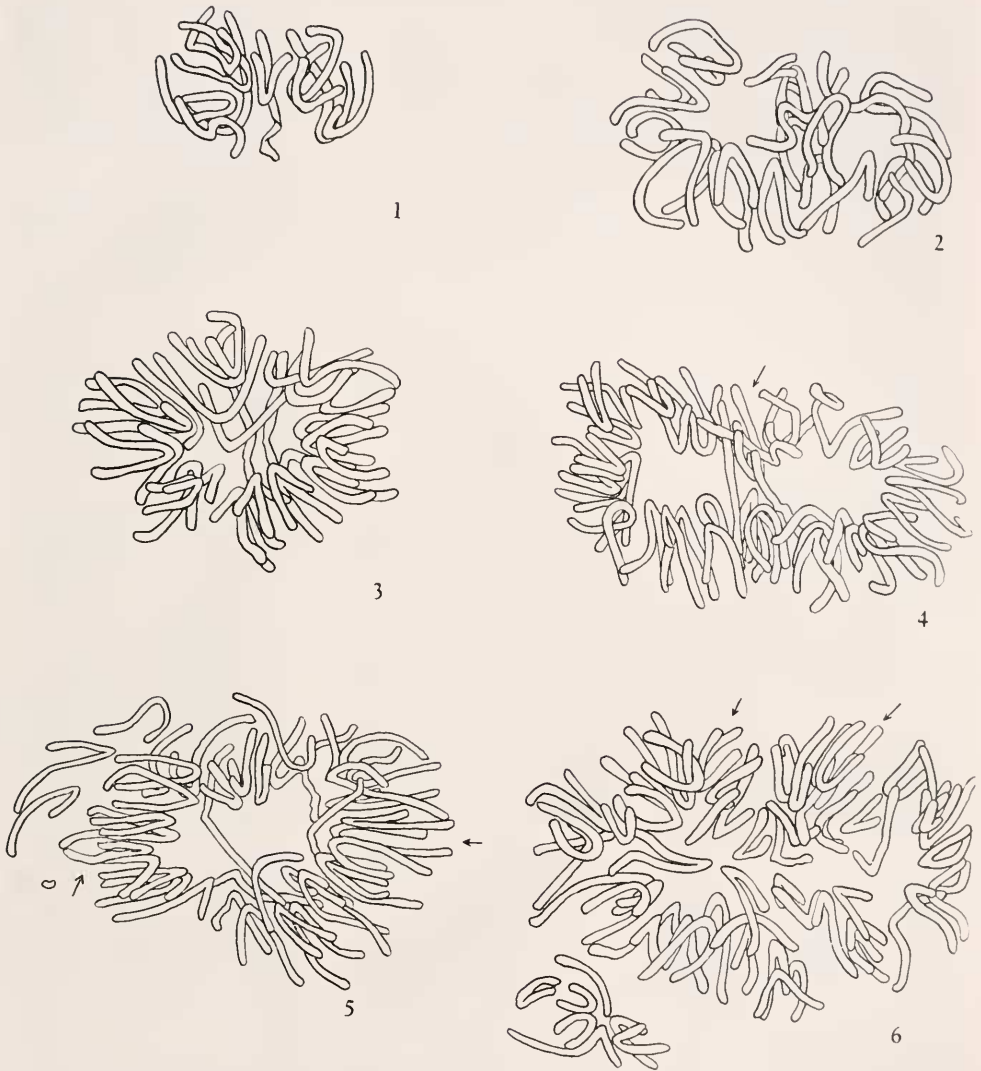
EXPLANATION OF FIGURES

FIGURES 1–6. Camera lucida drawings of representative metaphase figures in tail-tips of *T. torosus* larvae subjected to shipment. The arrows indicate regions where additional chromosomes were present but too obscure to be traced along their entire length. Figure 1: Haploid; 11 chromosomes counted. Figure 2: Diploid; 22 chromosomes counted. Figure 3: Triploid; 30 chromosomes counted. Figure 4: Tetraploid; 42 chromosomes counted. Figure 5: Pentaploid; 46 chromosomes counted. Figure 6: Octaploid; 65 chromosomes counted. Drawn at a magnification of 2100 ×, reduced by engraver to 1250 ×.

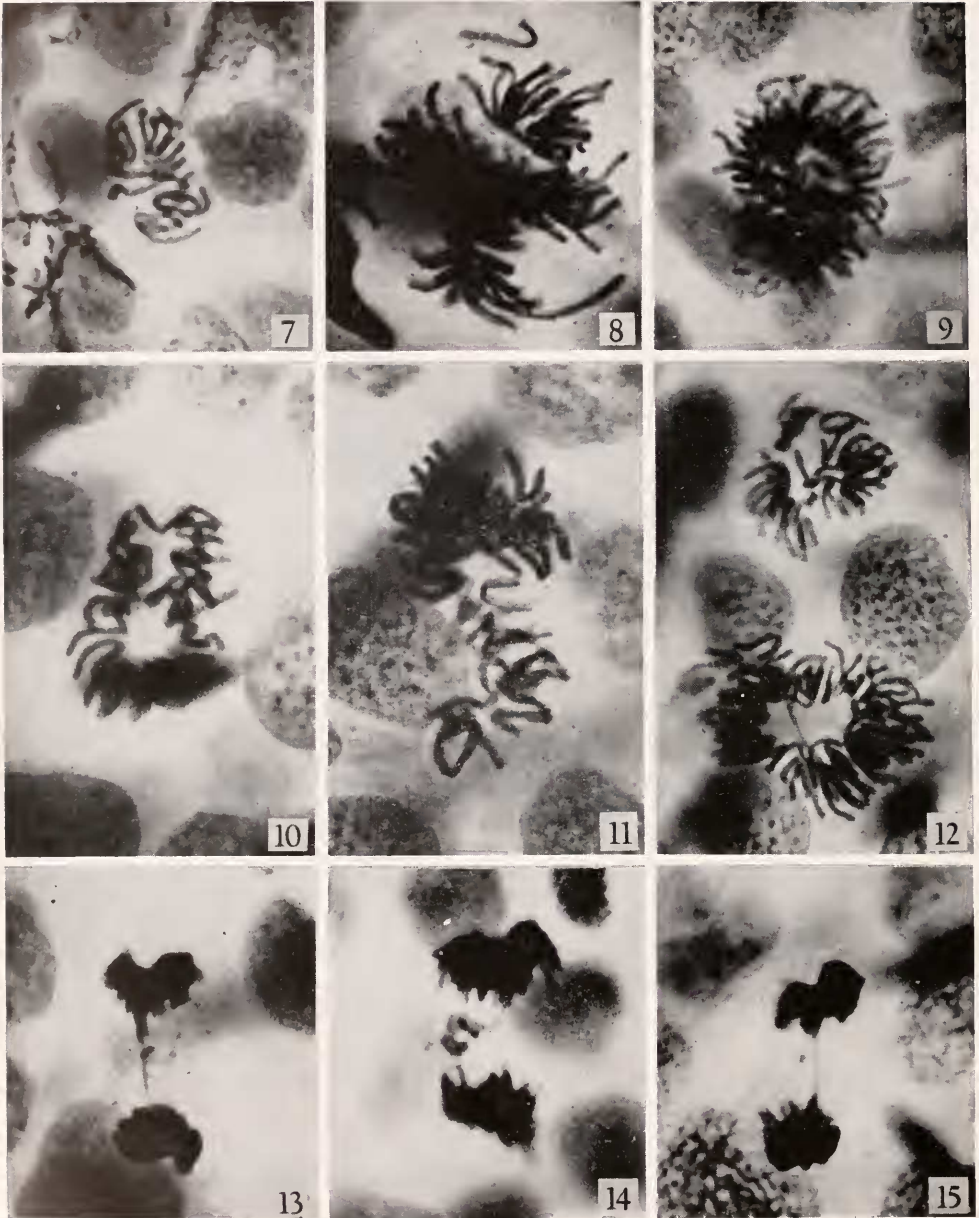PLATE I

Photomicrographs of mitotic figures from tail-tips of shipped *T. torosus* larvae. Magnifications: Figures 7, 8, 14: 1100 ×; Figures 9, 12: 960 ×; Figures 10, 11, 13, 15: 1160 ×. Reduced 20% off.

FIGURE 7.   Haploid metaphase (same as Fig. 1), with 11 chromosomes counted.

FIGURE 8.   Pentaploid metaphase, with 46 chromosomes counted. Most of the chromosomes in the figure are split.

in the 1947 and 1948 shipped material is higher than in the control material; the number of mosaics is notably higher.

The most significant data are probably those concerned with the second tips, as indicated in Table I. Among the 338 second tips clipped from the shipped larvae in 1947, 142 (42.0%) were normal diploids, 81 (23.9%) had nuclei of variable size, 39 (11.5%) had abnormal mitoses and 76 (22.5%) were chromosomal mosaics. This is a remarkably high frequency of mosaicism, although it is lower than that reported by us in preliminary notes for much smaller random samples of these same tips (Costello and Henley, 1947a, 1947b, 1948). Of the 269 second tips studied from the 1948 shipped material, 98 (36.4%) were normal diploids, 107 (39.8%) had nuclei of variable size, 55 (20.4%) had abnormal mitoses, and 9 (3.3%) were chromosomal mosaics. The percentage occurrence of aberrations when the 1947 and 1948 groups are taken as a whole is obviously considerably greater than that recorded for second tips from control larvae fixed in 1948. Especially striking is the 14.0% incidence of chromosomal mosaicism, as compared with 0.5% for the control animals.

Mosaicism. A wide variety of types of mosaics was found. Among the 1947 first tips from shipped larvae, there were four haploid/diploids, one diploid/hyperdiploid, four diploid/triploids, and one diploid/tetraploid. Eight of these 10 mosaics were confirmed by exact chromosome counts. In all, 55 of the 76 cases of mosaicism observed among the 1947 shipped second tips were checked by direct chromosome counts. The 76 mosaics included the following: one haploid/diploid; four hypodiploid/diploids; two hypodiploid/diploid/hyperdiploids; one hypodiploid/diploid/tetraploid; 20 diploid/hyperdiploids; 13 diploid/triploids; 19 diploid/tetraploids; three diploid/pentaploids; four diploid/hexaploids; one diploid/octaploid; one diploid/triploid/tetraploid; four diploid/tetraploid/pentaploids; one diploid/tetraploid hexaploid; one diploid/triploid/tetraploid/pentaploid; one diploid/hyperdiploid/tetraploid. Tail-tips of many of these complex mosaics showed some abnormal mitoses.

Nine chromosomal mosaics were found in the group of second tips from the 1948 shipped larvae, and 5 of these 9 were checked by chromosome counts; among them were one haploid/diploid; one diploid/hyperdiploid; 5 diploid/triploids; one diploid/tetraploid; and one diploid/pentaploid.

There were 11 cases of heteroploid figures in third tips of the 1947 material; two were checked by direct chromosome counts. The remainder were established by estimates of the number of chromosomes present in figures too crowded for exact

FIGURE 9. Possible hexaploid metaphase. The figure was too crowded for an accurate chromosome count to be practicable.

FIGURE 10. Hypodiploid metaphase. Twenty chromosomes were counted, and the figure was sufficiently well-spread that this count is probably reliable.

FIGURE 11. Triploid metaphase, with 32 chromosomes counted.

FIGURE 12. Diploid (upper) and pentaploid (lower) (same as Fig. 5) metaphases in adjacent epidermal cells. There were 46 chromosomes counted in the pentaploid figure.

FIGURE 13. "Sticky" anaphase, with deleted fragment and bridge.

FIGURE 14. Anaphase with four lost chromosomes or chromosome fragments. At least two of these deleted elements appear to be complete or nearly complete chromosomes.

FIGURE 15. "Sticky" anaphase with a deleted chromosome fragment attached by a very fine strand of chromatin to the upper group of chromosomes.

counting.   Of these 11 mosaics, one was hypodiploid/diploid; four were diploid/triploid; four were diploid/pentaploid; and two were diploid/hexaploid.   The single mosaic found in the third tips from the 1948 shipped larvae was hypodiploid/diploid.

All aneuploid mosaic constitutents listed above were established by chromosome counts, since such counts are the only reliable method for ascertaining chromosome number in metaphases where only one or two chromosomes have been added to or lost from the diploid chromosome complement.

The bizarre combinations of chromosome numbers were among the striking features of the complex mosaics.   In almost every case, the heteroploid metaphases occurred as isolated figures in single cells, rather than in patches of heteroploid tissue.   Similarly, the sporadic occurrence of very large interphase nuclei among nuclei of normal size is evidence that, in general, the mosaic cells are not present as sizable areas of tissue.   Larvae possessing these features can be characterized as predominantly diploid, but with some heteroploid cells making them mosaics.

Nuclear abnormalities.   Many of the interphase nuclei showed evidence of earlier mitotic abnormality by the presence of blebs of extruded chromatin, "amoeboid" lobes, or slender connecting bridges between two adjacent nuclei.   Quite frequently the periphery of the nucleus stained intensely with haematoxylin, while the central region was more nearly homogenous.   Comparable configurations have been reported by us as occurring in control larvae collected from very cold water (see Fig. 17 in the paper by Costello and Henley, 1949), and in experimentally cold-treated larvae (Henley, 1950).

Mitotic abnormalities.   A remarkable number of multipolar spindles was observed in tail-tips from the larvae subjected to shipment; examples are shown in

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PLATE II

Photomicrographs of epithelial nuclei and mitoses from tail-tips of shipped *T. torosus* larvae. Magnifications: Figure 16: 1100 ×; Figure 17: 560 ×; Figure 23: 960 ×; all other figures: 1160 ×.   Reduced 20% off.

FIGURE 16.   Nuclear débris resulting from the breakdown of epithelial nuclei.   The dark globules are intensely basophilic.   Small areas of such débris ("necrotic areas") are occasionally found in tail-tips from control larvae, but are not usually so extensive nor so frequent in occurrence as in shipped and experimentally cold-treated animals.

FIGURE 17.   Cell boundaries in the tail-tip epithelium.   This preparation was made utilizing a silver impregnation method, and the nuclei are not stained.   The characteristic shape of the cells is clearly evident, as are quite pronounced variations in cell size.

FIGURE 18.   Tripolar configuration of chromosomes.   Superficially, this figure appears to be in anaphase, but the morphology of the chromosomes appears to be more typical of metaphase.   A total of 18 chromosomes was counted.   The outline of the cell boundary is quite clearly defined.

FIGURE 19.   Tripolar telophase.   A fine connection is present between the two upper groups of chromosomes.

FIGURE 20.   Tetrapolar figure, displaced around a highly vacuolated cell.   The necrotic nucleus of this vacuolated cell is visible in the center of the clear area.

FIGURE 21.   Tripolar anaphase, with several persistent bridges between the two lower groups of chromosomes.   The dark granules at the bottom of the photomicrograph are nuclear débris.

FIGURE 22.   Tripolar anaphase, similar to that shown in Figure 18, but representing a slightly later stage.   Nineteen chromosomes were counted.

FIGURE 23.   Tetrapolar anaphase, in which a total of 75 chromosomes was counted.   One ring chromosome is visible at the right.

PLATE II
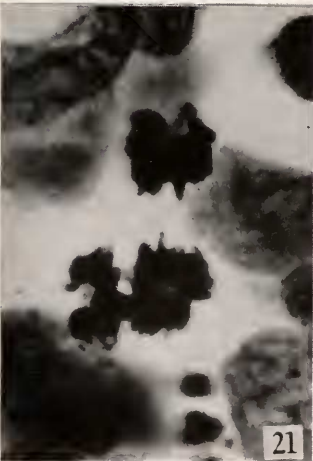
Figures 18–21 and Figure 23.   Seven clear tripolar anaphases and telophases, and 10 tetrapolar spindles were found on first, second and third tail-tips of the 1947 and the 1948 shipped material.   No such configurations have been found in tail-tips from control larvae of this species.

Another commonly observed type of aberration was the presence of relatively uncondensed masses of chromatin.   Similar configurations have been reported in experimentally cold-treated *T. torosus* larvae (Henley, 1950), and are also occasionally found in control larvae, especially those collected from very cold water (Costello and Henley, 1949).   These chromatin masses resemble abortive prophases.   They were sometimes noted near metaphases and appeared to represent chromatin material normally present as chromosomes in a diploid figure.

A number of types of abnormality were observed in metaphases.   These included "ragged" figures, in which the chromosomes were slender and thread-like rather than compact and cylindrical; the arrangement of the chromosomes on the spindle in these cases was often irregular.   The chromosomes in some metaphases were intensely basophilic and arranged in dense tangled masses very unlike the normal type.   Almost invariably these "wreath" configurations were too obscure for chromosome counts to be practicable.   A third abnormality observed in metaphase figures was the reversal of one or more chromosomes on the spindle, so that the kinetochore region was directed outward from the center of the ring-shaped figure, rather than toward the center as is normally the case.   This condition may foreshadow another frequently observed abnormality, in which the chromosomes had become completely lost from the metaphase spindle and were apparently loose in the cytoplasm.   Such "lost" chromosomes or chromosome fragments have been observed in a few of the control preparations (Costello and Henley, 1949), but are not nearly so common there as in these shipped larvae.

Many types of anomalies were observed in anaphase and telophase mitoses, including the multipolar spindles referred to above.   Sometimes the two poles of bipolar figures were quite markedly asymmetric, so that one had discernibly more chromatin material than the other.   There were also bent spindles, in which the center of orientation of one of the two poles was almost at right angles to that of the other.   "Sticky" anaphases and telophases were frequently encountered (Figs. 13, 15), as well as chromosomes and chromosome fragments which appeared to have lost their spindle connections, so that they were left behind in the migration of the daughter elements toward the poles.   An extreme example of this type is shown in Figure 14.   The loss or disorientation of chromosomes at the poles of anaphase or telophase figures was quite common.   In a few instances, anaphases were observed in which large unorganized masses of chromatin material were present on the spindle.

Structural abnormalities of the larvae.   The process of shipment appears in some way to have affected the formation of the lateral line organs in these larvae; many cases were found in which displacement of the lateral line condensations of the tail-tip had occurred, similar to those reported for experimentally cold-treated *T. torosus* larvae (Henley, 1950).   Often supernumerary condensations were present toward the edges of the tail-fins; these appeared to be morphologically identical with the normal lateral line organs.   The significance of these developmental anomalies is thus far obscure.

There was no observable correlation between the general morphology of the larvae and the presence or absence of cytological abnormality. A careful check of the photographs of the larvae indicated no recognizable basis for identifying larvae with either abnormal mitoses or the described types of mosaic cells. This confirms observations reported by us for control *T. torosus* larvae, as well as for experimentally cold-treated animals.

DISCUSSION

I. Possible factors involved in the production of abnormalities

The foregoing results indicate clearly that a higher percentage of various nuclear and mitotic abnormalities is found in shipped than in control material. The major question for discussion is concerned with the reasons for such an effect. Among the possible factors contributing to the production of the abnormalities observed in the shipped material, the following may be considered:

a) *Cold.* The eggs were shipped with ice in the thermos jugs, and the temperature of the water presumably remained quite low for an extended period of time. Experimental cold-treatment has been demonstrated to produce similar anomalies in larvae of *Triturus torosus* (Henley, 1950), as well as in other species of salamanders (Böök, 1943, 1945; Fischberg, 1947; *et al.*).

b) *Partial anaerobiosis.* Utilization of most of the available oxygen within the closed jugs could have occurred during the one-week period of transit. This deficiency of oxygen might be especially pronounced near the centers of the jelly masses. There is some evidence to indicate that anoxia is of considerable importance in producing cytological abnormality in tail-tips of *Amblystoma punctatum* larvae (unpublished data).

c) *Accumulation of carbon dioxide.* The gaseous products of metabolism of the embryos do not escape from the closed jug system, from the time of shipment until the time of receipt. Such accumulation of carbon dioxide may have deleterious effects on the embryos, either directly or indirectly, through lowering of the pH of the medium.

d) *Mechanical agitation.* Shipment by Railway Express probably involves almost continuous shaking of the jug contents.

e) *Toxic water.* The ice used was made by freezing ordinary tap water; even though the liquid medium was non-chlorinated lake water (also used for raising the control animals), the water from the melting ice may have introduced toxic factors. In general, it is the experience of the Stanford group of embryologists that larvae which are raised in heavily chlorinated or other unsuitable water are characterized by laterally-curled tail-tips, with deviated tail-fins. No such curled tails were noted in our material, so perhaps this factor may be eliminated from further consideration.

f) *Irradiation.* This remote possiblity is mentioned only because of the similarity between the observed aberrations and the types of irradiation-produced anomalies described by Alberti and Politzer (1924a, 1924b). We have no evidence that any such radiations were present, and it would be a remarkable coincidence if they were operative, even in different dosages, during two separate salamander breeding seasons.

g). Anaesthesia and other factors inherent in the process of photographing the larvae. Theoretically, the anomalies found might be attributed to the effects of the anaesthetic required to immobilize the larvae for photography, or to the heat given off by the Photoflood lamps used as a light source. Such effects, if present, would be expected to bear a definite relation to the time of subsequent clipping. However, they can probably be ruled out, on the basis of cytological studies of tail-tips from *Amblystoma punctatum* larvae which were not subjected to anaesthesia or photography (unpublished data).

II. The correlation between cytological abnormality present on first, second and third tail-tips

On the assumption that the factors responsible for mitotic anomalies were present during the period of egg shipment, as indicated above, the data were examined to ascertain the degree of correlation between the occurrence of abnormalities in first, second and third tail-tips. If the factors producing abnormality were operative during the period of late cleavage, gastrulation and neurulation, we should expect to find some regularity in the subsequent events taking place in the epidermal cells of the growing larvae. There are several possibilities: 1) All three tail-tips of an affected larva might have a similar high percentage of abnormal figures. 2) The three tail-tips could exhibit a progressive increase in the number of abnormal figures, due to an increase in the number of cell progeny of the affected cells. 3) There might be a progressive decrease in the number of atypical mitoses in the three tips. This would obtain if the abnormal cells ceased to divide further and, becoming necrotic, were eliminated from the tip.

The data indicate that very little correlation exists. Only three second tail-tips were mosaic among the 23 larvae whose first tips were characterized by that condition. From this same group of 23, not even one third tail-tip was found to have heteroploid metaphases. Of the 85 larvae with mosaic second tips, three belonged to the group of 23 with mosaic first tips. A single animal of the group of 85 showed mosaicism in its third tail-tip. Only one individual of the 12 which had mosaic third tips was demonstrated to have had heteroploid figures present before the time of the third clipping.

In addition to this lack of correlation, there was no progressive increase or decrease in abnormality. Instead, the incidence of atypical figures was found to be much higher in second tips (85 mosaics) than in "firsts" (23) or "thirds" (12). How can this be explained?

The second tips are by far the most favorable material, for several reasons. They are relatively free of yolk, whereas the first tips may have some yolk-obscured cells, especially if clipped a little too early. Furthermore, the incidence of mitosis is very much higher in the second tips than in either "firsts" or "thirds." This may be due to the onset of a period of rapid growth and cell multiplication concurrent with the beginning of feeding; such a period presumably tapers off after about 10 days. Another possibility is that there is more rapid cell division following the stimulation of the initial tail-clipping, which persists for as much as 12 to 14 days. However, if this be true, it seems strange that a similar stimulation of mitosis does not follow the second amputation. Finally, one must consider the fact that the second tips have noticeably fewer pigment cells than the "firsts" (but not

fewer than the "thirds"), so that a smaller number of figures would be obscured. This finding, in itself, obviously does not account for the differences between the "seconds" on the one hand, and the "firsts" and "thirds" on the other.

The frequent occurrence in this material of necrotic areas, with nuclear débris resulting from partial or complete disintegration of epithelial nuclei, is an established fact. A typical example of this phenomenon is figured (Fig. 16). The staining characteristics of such areas are described briefly by Costello and Henley (1949). We have no way of knowing whether or not nuclear necrosis is initiated in abnormal cells. It appears that arrested nuclear disintegration could be an effective cause of hypodiploid nuclei.

It must be concluded that, at the present time, we cannot adequately account for the high percentage of deviations from normal mitotic conditions. Neither can we account for the higher incidence of abnormalities found in the 1947 shipped material, as compared with the larvae subjected to shipment in 1948. We do know, however, that the 1948 embryos were kept under more carefully controlled conditions before shipment. If the radical aberrations described herein are the result of such minor environmental changes as obtain in the ordinary procedures for handling and shipping embryological materials, it is of vital importance to emphasize this fact. Many amphibian embryologists working in this country today are experimenting with cytologically sensitive and perhaps abnormal material.

Summary

1. Tail-tips from *Triturus torosus* larvae subjected to shipment by Railway Express have been studied cytologically.

2. A total of 838 "first" tips was examined. Of these, 381 (45.5%) were normal diploids, 359 (42.8%) had nuclei of variable size and/or variations in nucleolar number, 75 (8.9%) had abnormal mitoses, and 23 (2.7%) were chromosomal mosaics.

3. Of the 607 regenerated "second" tips studied, 240 (39.5%) were normal diploids, 188 (30.9%) had nuclei of variable size and/or variations in nucleolar number, 94 (15.5%) had abnormal mitoses, and 85 (14.0%) were chromosomal mosaics.

4. Three hundred fifty-six second regenerates ("third" tips) were examined. Among these, 153 (43.0%) were normal diploids, 159 (44.6%) had nuclei of variable size and/or variations in nucleolar number, 32 (9.0%) had abnormal mitoses, and 12 (3.4%) were chromosomal mosaics.

5. The following types of mosaics were found: haploid/diploid; hypodiploid/diploid; hypodiploid/diploid/hyperdiploid; hypodiploid/diploid/tetraploid; diploid/hyperdiploid; diploid/triploid; diploid/tetraploid; diploid/pentaploid; diploid/hexaploid; diploid/octaploid; diploid/triploid/tetraploid; diploid/tetraploid/pentaploid; diploid/tetraploid/hexaploid; diploid/triploid/tetraploid/pentaploid; diploid/hyperdiploid/tetraploid. These mosaics were predominantly diploid, with, for the most part, isolated heteroploid cells in scattered areas.

6. Seventeen instances of multipolar anaphases and telophases were found in the tail-tips from these shipped larvae. Seven of these were tripolar and 10 were tetrapolar.

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